



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P1375PC01	FOR FURTHER ACTION		See Form PCT/PEA/416
International application No. PCT/EP2005/000444	International filing date (day/month/year) 13.01.2005	Priority date (day/month/year) 14.01.2004	
International Patent Classification (IPC) or national classification and IPC C12N9/52, C12N15/62, C12N15/57, C07K16/46, C12N15/63, C12N1/21, C12P21/02			
Applicant CONSEJO SUPERIOR DE INVESTIGACIONES CIENT... et al			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input type="checkbox"/> sent to the applicant and to the International Bureau) a total of sheets, as follows:</p> <p><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand 07.11.2005		Date of completion of this report 02.02.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer Seroz, T Telephone No. +49 89 2399-7789 	

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

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Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-28 as originally filed

Sequence listings part of the description, Pages

1-6 as originally filed

Claims, Numbers

1-34 as originally filed

Drawings, Sheets

1/9-9/9 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	5,12,13
	No: Claims	1-4,6-11,14-34
Inventive step (IS)	Yes: Claims	
	No: Claims	1-34
Industrial applicability (IA)	Yes: Claims	1-34
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

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Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

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(SEPARATE SHEET)**

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Additional remarks to item I

This first written opinion was established on the application documents as filed and the written sequence listing pages 1-9, SEQ ID No 1 to SEQ ID No 10.

Additional remarks to item V

1. The present application discloses a gene construction comprising an expression cassette coding for a signal peptide, a single-domain recombinant antibody and the C-terminal domain of an autotransporter. Such a construct is used for anchoring and expressing a single-domain recombinant antibody on the surface of the outer membrane of a bacteria.

The following document is referred to in the present written opinion:

D1: VEIGA ESTEBAN ET AL: "Neutralization of enteric coronaviruses with *Escherichia coli* cells expressing single-chain Fv-autotransporter fusions." JOURNAL OF VIROLOGY, vol. 77, no. 24, December 2003 (2003-12), pages 13396-13398, XP002328574 ISSN: 0022-538X

D2: VEIGA E ET AL: "PROBING SECRETION AND TRANSLOCATION OF A BETA-AUTOTRANSPORTER USING A REPORTER SINGLE-CHAIN FV AS A COGNATE PASSENGER DOMAIN" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 33, no. 6, September 1999 (1999-09), pages 1232-1243, XP001007312 ISSN: 0950-382X

Claim 4 relates to a construct comprising two or more, equal or different, single-domain recombinant antibodies.

Document D1 relates to the construction of a vector that contains an expression cassette, said cassette comprising a *lac* promoter operably linked to a signal peptide PelB, a DNA sequence coding for a single chain Fv protein (V_H-V_L fusion), an E-tag epitope and the C-terminal transporter domain of the IgAP from *Neisseria gonorrhoeae*. This construct is used for transforming *Escherichia coli* cells (gram-negative bacteria) in order to express a single chain recombinant antibody on the surface of the outer-

membrane of said bacteria.

Thus, the construct of D1 contains two different single-domain recombinant antibodies (V_H - V_L).

Therefore, D1 anticipates the subject matter of claim 4.

Claim 4 depending on claim 1, this latter includes the construct disclosed in claim 4 and is, as a consequence, not novel over D1.

Hence, in the light of the prior art document D1, it seems that the subject matter disclosed in **claims 1-4, 6-11, 14-34** is not novel (Article 33(2) PCT).

In addition, **claims 5, 12 and 13** do not contain additional technical features which taken alone or in combination with those of the claims to which they refer could render the claimed gene construction inventive over D1 (Article 33(3) PCT).

2. Moreover, a signal peptide can be an nuclear localization signal (NLS) of a protein expressed in eucaryotic cells. In that particular case, the technical problem underlying the present application is not solved.

Therefore, the scope of the claims has to be restricted to signal peptides involved in the passage of the recombinant protein across the cell membrane.

Furthermore, as mentioned on page 13 of the present application, it may be used the C-terminal domain of any autotransporter capable of translocating passenger domain to which it is bound. The scope of the claims has to be limited to said autotransporter capable of translocating passenger domain, the absence of such a limitation resulting in a lack of inventive step. Indeed, autotransporter that are not capable of translocating the single-domain recombinant antibody do not solve the technical problem underlying the present application.

3. The subject matter of claims 1-34 seems to satisfy the conditions of Article 33(4) PCT.